JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1988, p. 213–215
0095-1137/88/020213-03$02.00/0

Vol. 26, No. 2

Comparative Study of Inactivation of Herpes Simplex Virus Types 1 and 2 by Commonly Used Antiseptic Agents

WENDY S. CROUGHAN† AND ABBAS M. BEHBEHANI*

Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, Kansas 66103

Received 1 June 1987/Accepted 26 October 1987

A comparative study of the different reactions of herpes simplex virus types 1 and 2 to Lysol, Listerine, bleach, rubbing alcohol, Alcide disinfectant (Alcide Corp., Westport, Conn.), and various pHs, temperatures, and UV light exposures was performed. Both types of stock virus (titers of approximately 10^6 and $10^{5.5}$ for types 1 and 2, respectively) were inactivated by 0.5% Lysol in 5 min; by Listerine (1:1 mixtures) in 5 min; by 2,000 ppm (2,000 μl/liter) of bleach in 10 min; by rubbing alcohol (1:1 mixtures) at zero time; by Alcide disinfectant (0.2 ml of virus plus 2.0 ml of Alcide) at zero time; by pHs 3, 5, and 11 in 10 min; and by a temperature of 56°C in 30 min. A germicidal lamp (model G30TB; General Electric Co., Schenectady, N.Y.) (30 W) at a distance of 48 cm failed to completely inactivate the two types in 15 min. Type 1 showed slightly more resistance to Listerine and bleach and significantly more resistance to heat; moreover, pH 9 did not affect the infectivity of either type after 10 min.

Herpes simplex virus is one of the most ubiquitous human pathogenic viruses. Infection with this virus occurs at an early age, and about one-third of the U.S. population has at least one recurrent herpetic infection (genital or nongenital) per year. A number of reports indicate that the two types of this virus (type 1, involved mainly in infections of skin, mouth, eye, and brain, and type 2, involved mainly in genital and congenital infections) differ from one another in certain biological properties, such as resistance to heat (6). However, no comprehensive study of the comparative resistance of the two types to various commonly used antiseptic agents has been done. Here we report the results of a comparative study of the reactions of the two types to Lysol, Listerine, bleach, rubbing alcohol, and a newly marketed disinfectant, Alcide (Alcide Corp., Westport, Conn.). We also studied the different effects of various pHs, temperatures, and UV light exposures on the two types of virus.

MATERIALS AND METHODS

Viruses. The two types of virus (strains Patton [type 1] and 333 [type 2]) were kindly provided by Fred Rapp, Pennsylvania State University, Hershey. They were grown in 32-oz (907.2-g) bottles of Vero cells maintained in complete Eagle minimum essential medium containing 3% fetal bovine serum at 37°C. When 90% of the cells showed cytopathic effects, they were frozen and thawed three times and then harvested into large pooled virus stocks. The two stock virus preparations were clarified by centrifugation, distributed in 2- and 5-ml portions, and preserved at −70°C until used. Tube cultures of Vero cells were similarly prepared and used for the titration of virus infectivity in all test and control experiments. Titration was done by serial dilution from 10^{-1} to 10^{-6} and inoculation in 0.1-ml amounts of each dilution into three or four tube cultures; the approximate titer was calculated by the Reed-Muench method as described previously (1). Samples of the two virus types had a pH of around 7.2 and titers of around 10^6 (type 1) and $10^{5.5}$ (type 2) per 0.1 ml in Vero cell tube cultures. The two virus types were titrated simultaneously in all test experiments as controls. All undiluted mixtures of viruses and antiseptic agents, with the exception of Listerine-virus mixtures, were toxic for cell cultures; granulation of cells and sloughing off the glass surface occurred within 18 h.

Commercial Lysol. Lysol was diluted with Hanks balanced salt solution to obtain 1, 0.5, 0.25, and 0.1% dilutions. Equal volumes of these dilutions and virus types were mixed, and the mixtures, kept at 25°C, were titrated after 5 and 10 min.

Commercial Listerine. Equal volumes of Listerine and virus types were mixed, and the mixtures were titrated immediately and after 1 and 5 min at 25°C. In contrast to the other antiseptic agents, 1:1 mixtures of virus types and Listerine were not toxic for cell cultures and hence were also tested for viral infectivity.

Commercial bleach. Bleach was diluted with Hanks balanced salt solution to obtain 100, 200, 500, 1,000 and 2,000 ppm (microliters per liter) of sodium hypochlorite (200 ppm is recommended for laundering by the manufacturer). Equal volumes of these dilutions and virus types were mixed, and the mixtures were titrated after 10 min at 25°C.

Commercial rubbing alcohol (70% isopropyl alcohol). Equal volumes of virus types and rubbing alcohol, brought up to 25 and 37°C, were mixed, and the mixtures were titrated immediately and after 1 and 5 min.

Alcide disinfectant. The two components of Alcide were mixed, and the product was mixed with virus types as recommended by the manufacturer (i.e., 0.2 ml of virus plus 2 ml of disinfectant). The mixtures were titrated immediately and after 1 and 3 min at 25°C. The prepared disinfectant was kept at room temperature for 14 days, after which it was tested again as described above.

pH. Samples of virus types were adjusted to pHs 3, 5, 9, and 11 with 0.1 N HCl or 0.1 N NaOH, kept at 25°C, and titrated after 10 min.

UV light. A germicidal lamp (model G30TB; General Electric Co., Schenectady, N.Y.) (30 W) installed inside a tissue culture enclosure (model 1100; Labconco, Kansas City, Mo.) was used. A 2-ml sample of virus, evenly spread in a petri dish (60 by 15 mm), was placed at a distance of 48 cm under the UV light (average distance between the UV light bulb and the work surface area inside the enclosure) for

* Corresponding author.
† Present address: Hazelton Laboratories, Lenexa, KS 66215.

TABLE 1. Effect of Lysol on the infectivity of herpes simplex virus types 1 and 2

Virus (titer of control virus)	Virus titer (log_{10} TCD_{50}[a]/0.1 ml) after treatment with the indicated concn of Lysol at 25°C for:							
	5 min				10 min			
	1%	0.5%	0.25%	0.1%	1%	0.5%	0.25%	0.1%
1 ($10^{6.25}$)	<1	<1	2.5	4.5	<1	<1	1.5	4
2 ($10^{5.5}$)	<1	<1	<1	3.5	<1	<1	<1	3.5

[a] TCD_{50}, 50% Tissue culture infective dose.

TABLE 2. Effect of commercial bleach on the infectivity of herpes simplex virus types 1 and 2

Virus type (titer of control virus)	Virus titer (log_{10} TCD_{50}[a]/0.1 ml) after treatment with the indicated concn of bleach for 10 min at 25°C:				
	100 ppm	200 ppm	500 ppm	1,000 ppm	2,000 ppm
1 (10^{6})	5.5	6	5.5	3.5	<1
2 ($10^{5.75}$)	5	5	4.5	<1	<1

[a] See Table 1, footnote *a*.

30 s and 1, 2, 3, 5, 10, and 15 min. The depth of the virus suspension in the petri dish was about 1.5 mm.

RESULTS

The effects of Lysol and bleach on the infectivity of herpes simplex virus types 1 and 2 are shown in Tables 1 and 2, respectively. Table 3 shows the effects of various temperatures and pHs, and Table 4 shows the effects of UV light.

Lysol at 1.0 and 0.5% concentrations reduced the titers of both types to $<10^1$ in 5 min at 25°C. At a 0.25% concentration, the titer of type 1 was reduced to $10^{2.5}$ in 5 min and to $10^{1.5}$ in 10 min, while this concentration reduced the titer of type 2 to $<10^1$ in 5 min. A concentration of 0.1% caused 1.75- and 2.25-log decreases in the titers of type 1 in 5 and 10 min, respectively, and a 2-log decrease in the titers of type 2 in 5 and 10 min (Table 1).

Listerine inactivated both types completely in 5 min at 25°C. However, after 1 min, a 1:1 mixture of type 1 and Listerine showed infectivity when diluted up to 1:8, while a 1:1 mixture of type 2 and Listerine showed no infectivity. At zero time, a 1:1 mixture of type 1 and Listerine had a titer of 10^1, and that of type 2 and Listerine showed infectivity when diluted up to 1:8.

Bleach reduced the titers of both types to $<10^1$ at a concentration of 2,000 ppm but did so only with type 2 at a concentration of 1,000 ppm after 10 min at 25°C. Concentrations of 500, 200, and 100 ppm, however, had a much less significant effect on both types (a 0.75- to 1.25-log reduction) (Table 2).

Rubbing alcohol reduced the titers of both types to $<10^1$ at 25 and 37°C at zero time and after 5 min.

Alcide disinfectant tested both immediately following preparation and after storage at 25°C for 14 days reduced the titers of both types to $<10^{-1}$ at zero time and after 1 and 3 min.

The titers of both types were reduced to $<10^1$ when the virus suspension was adjusted to pHs 3, 5, and 11 and kept at 25°C for 10 min. However, pH 9 did not affect the titers of either type under the same conditions (Table 3).

No significant loss of infectivity of the two types was observed after 10 h at 25°C and after 18 h at 37°C. However, at 56°C the titer of type 1 decreased to $<10^1$ after 30 min, while the titer of type 2 decreased to $<10^1$ after 10 min (Table 3). Moreover, at 56°C there was a 2-log reduction in the titers of both types after 5 min.

UV light at a distance of 48 cm affected both types equally, and the titers were reduced by 50% after exposure for 5 min. Exposure for 15 min reduced the titers of types 1 and 2 to $10^{1.5}$ and 10^1, respectively (Table 4).

DISCUSSION

A search of the literature revealed little information on the different reactions of herpes simplex virus types 1 and 2 to commonly used antiseptic agents. It is generally recognized that type 2 is more thermolabile than type 1; however, the thermal sensitivity of both types has been shown to be affected interdependently by both the composition and pH of the suspending medium (5). The virus is stabilized by molar Na_2SO_4 at 50°C for 1 h (7). Pasteurization (60°C for 10 h), UV light (model G8TS UV bulb [General Electric] for 4 min at a distance of 20 cm), and microwaves (4 min) destroy the virus (4, 6, 9). Moreover, both types are inactivated within

TABLE 3. Effect of various pHs and temperatures on the infectivity of herpes simplex virus types 1 and 2

Virus type (titer of control virus)	Virus titer (log_{10} TCD_{50}[a]/0.1 ml) after exposure to the following:							
	pH (after 10 min at 25°C):		Temp (°C):					
			25, after 8 and 10 h	37, after 8, 10, 14, and 18 h	56 after:			
	3, 5, and 11	9			5 min	10 min	20 min	30 min
1 ($10^{6.5}$)	<1	6	5.5	5.5	3.5	1	1	<1
2 ($10^{5.5}$)	<1	5.5	5	5	3.5	<1	<1	ND[b]

[a] See Table 1, footnote *a*.
[b] ND, Not done.

TABLE 4. Effect of UV light on the infectivity of herpes simplex virus types 1 and 2

Virus type (titer of control virus)	Virus titer (log_{10} TCD_{50}[a]/0.1 ml) after exposure to UV light[b] for the indicated times at 25°C:						
	30 s	1 min	2 min	3 min	5 min	10 min	15 min
1 (10^6)	5	5	3.5	2.5	3.0	3.0	1.5
2 ($10^{5.75}$)	5	4	3.5	3	2.5	1.5	1

[a] See Table 1, footnote *a*.
[b] The germicidal lamp model (G30TB; General Electric) (30 W) was located 48 cm from the virus suspension.

24 h when treated at 37°C with 1 mg of copper-catalyzed sodium ascorbate per 1 ml of virus suspension (8). However, after inactivation by heat (56 or 70°C), both types were shown to be infectious for certain cell cultures (e.g., BHK-21) by transfection by the calcium-dimethyl sulfoxide technique (3), and after inactivation by UV light, both types were shown to be capable of biochemically transforming mouse cells (2).

We followed the recommendations of the manufacturers in testing the antiviral activity of the commonly used antiseptic agents included in this study. Lysol and bleach were tested at several dilutions to establish the endpoints for their antiviral activities. All virus suspensions of the two types used in the tests were made in the same suspending medium at a pH of around 7.2. In the UV light experiment, the virus was kept at a distance of 48 cm, the average when work with viruses is performed inside the specified tissue culture enclosure described above.

The results reported here indicate that the two types are equally susceptible to Lysol and that a 0.5% concentration of this agent reduces the titers of both types to $<10^1$ in 5 min at 25°C. Listerine in a 1:1 mixture with either type at 25°C destroyed the virus in 5 min; however, after 1 min, there was some residual infectivity in the Listerine-type 1 mixture, revealing different reactions of the two types. Bleach did not affect the infectivity of either type at the recommended concentration (200 ppm) in 10 min at 25°C. However, at 2,000 ppm, the titers of both types were reduced to $<10^1$. At 1,000 ppm, the titer of type 1 was reduced to only $10^{3.5}$, while that of type 2 was reduced to $<10^1$, again revealing different reactions of the two types to this agent. Isopropyl alcohol (70%) at 25 and 37°C (as 1:1 mixtures) and Alcide disinfectant at 25°C (both freshly prepared and after storage at 25°C for 14 days as a mixture of 0.2 ml of virus plus 2.0 ml of Alcide) reduced the titers of the two types to $<10^1$ at zero time.

The reactions of the two types to various pHs were quite remarkable. While pHs 3, 5, and 11 reduced the titers of both types to $<10^1$ in 10 min at 25°C, pH 9 did not affect the titers of either type under the same conditions.

The results of our comparative study of the inactivation of the two types by heat and UV light were similar to those reported by others (2, 6). Temperatures of 25 and 37°C did not affect the titers of either type significantly in 18 h. However, a temperature of 56°C reduced the titers of types 1 and 2 to $<10^1$ in 30 and 10 min, respectively, again revealing the widely reported different reactions of the two types to heat. UV light at a distance of 48 cm from virus suspensions did not completely destroy the infectivity of either type, even after 15 min; titers of $10^{1.5}$ and 10^1 were detected for types 1 and 2, respectively. This observation should be considered by those who use this type (or model) of tissue culture enclosure for virological work.

ACKNOWLEDGMENT

We thank Nancy J. Tower for her excellent secretarial assistance.

LITERATURE CITED

1. **Behbehani, A. M.** 1972. Laboratory diagnosis of viral, bedsonial and rickettsial diseases. Charles C Thomas, Publisher, Springfield, Ill.
2. **Duff, R., and F. Rapp.** 1971. Properties of hamster embryo fibroblasts transformed in vitro after exposure to ultraviolet-irradiated herpes simplex virus type 2. J. Virol. **8**:469–477.
3. **Fenyves, A., and L. Strupp.** 1982. Heat resistant infectivity of herpes simplex virus revealed by viral transfection. Intervirology **17**:228–239.
4. **Hilfenhaus, J., A. Herman, R. Mauler, and A. M. Price.** 1986. Inactivation of the AIDS-causing retrovirus and other human viruses in antihemophilic plasma protein preparations by pasteurization. Vox Sang. **50**:208–211.
5. **Lancz, G., and J. Sample.** 1985. Thermal-pH inactivation of herpes simplex virus: interdependence of the medium composition and the pH on the rate of virus inactivation. Arch. Virol. **84**:141–146.
6. **Rapp, F., and N. Turner.** 1978. Biochemical transformation of mouse cells by herpes simplex virus types 1 and 2: comparison of different methods for inactivation of viruses. Arch. Virol. **56**:77–87.
7. **Wallis, C., and J. L. Melnick.** 1965. Thermostabilization of herpesvirus. J. Bacteriol. **90**:1632–1637.
8. **White, L. A., C. Y. Freeman, B. D. Forrester, and W. A. Chappell.** 1986. In vitro effect of ascorbic acid on the infectivity of herpesviruses and paramyxoviruses. J. Clin. Microbiol. **24**:527–531.
9. **Young, S. K., D. C. Graves, M. D. Rohrer, and R. A. Bulard.** 1985. Microwave sterilization of nitrous oxide nasal hoods contaminated with virus. Oral Surg. Oral Med. Oral Pathol. **60**:581–585.